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## Research Article

### FORMULATION AND EVALUATION OF CREAM CONTAINING *CALENDULA OFFICINALIS* AND *CENTELLA ASIATICA* AQUEOUS EXTRACTS

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#### ABSTRACT

The objective of this study was preparing and evaluating cream formulations containing aqueous extracts of *Calendula officinalis* and *Centella asiatica*, and also to conduct a preliminary phytochemical analysis for the said aqueous extracts. The plant aqueous extracts were prepared by macerating the dry powders of the aforementioned plants; water-in-oil creams were prepared in varying concentrations of *Calendula officinalis* and *Centella asiatica* aqueous extracts (5%, 7.5% and 10%). The creams thus prepared varied in the colour and texture, but all had good physical characters for a cream. The preliminary phytochemical analysis shows that *Calendula officinalis* aqueous extract contains flavonoids, saponins and tannins, while *Centella asiatica* aqueous extract contains flavonoids and tannins. Based on the results we have concluded that the formulation containing 5% of aqueous had the best overall characteristics of a cream compared to the other formulations as it had a good colour, was smooth in texture and had better stability.

**Keywords:** *Calendula officinalis*, *Centella asiatica*, cream formulation.

#### INTRODUCTION

Creams are a type of dosage form which are prepared by incorporating an aqueous phase into an oil phase, or vice versa, to form an emulsion which is thick and semi-solid in nature. Based upon the nature of emulsion formed creams can be categorised as, oil-in-water (O/W) creams, where the oil phase is observed as minute globules within the aqueous phase (e.g., Vanishing cream) or, water-in-oil (W/O) creams, in which the aqueous phase is dispersed within the oil phase (e.g., cold creams).<sup>1</sup> Depending upon the intended use creams can be applied to various parts of the body; topical creams are those type of creams which are strictly meant for external use and are to be applied onto the skin to treat a dermal disease such as psoriasis. Creams are very popular within the general public as they are very convenient to use and easy to store.

The general populous prefer herbal products as they produce minimal side-effects compared to conventional products and this even extends to cosmetics and medicines. The use of herbs as drugs is an ancient practice most of which is lost through time. It is evident that the drugs derived from natural sources are just as effective as conventional alternatives with minimal toxic effects on the body.<sup>2</sup>

*Calendula officinalis*, more commonly known as pot Marigold, is a flowering plant with orange-yellow flowers, a commonly used medicinal plant which grows throughout Asia, Europe, and America. Evidence shows that it possesses wound healing, anti-inflammatory, antioxidant, sun protecting and moisturizing properties. The phytochemicals found to be present in the said plant are flavonoids, saponins, terpenoids, phenolic compounds.<sup>3</sup>

*Centella asiatica* belonging to family Umbellifere (Apiceae), grows throughout the Indian sub-continent and is commonly known in India, as Gotu Kola. It is found to possess various medicinal properties such as anti-inflammatory, anti-oxidant, wound healing, nephron-protective, anti-cancer properties. The primary phytoconstituents that have been found in the plants are Asiaticoside, asiatic acid, madecassoside and madasiatic acid, which are responsible for the said medicinal properties when tested in animal models.<sup>4</sup>

#### Objective

The aim of the present study is to prepare and develop a stable cream formulation containing the aqueous extracts of both the plants *Calendula officinalis* and *Centella asiatica* and to evaluate their physical parameters. Analysis of the phytochemicals present in the aqueous extracts was also conducted to determine the presence of the biologically active phytochemicals by conducting a preliminary phytochemical evaluation.

#### MATERIALS AND METHODS

**Procurement of Plant materials:** 100g dry powder of *Calendula officinalis* flower and 100g dry powder of *Centella asiatica* was purchased through e-commerce websites; sold, manufactured, and marketed by Saptraag Foundation, Shahdara, Delhi, India, and Biotic Nature Products, Kelapur - 445302, Maharashtra, India respectively.

**Authentication of Plant Materials:** The authentication of the plant powders of *Calendula officinalis* and *Centella asiatica* was done in Karavali College of Pharmacy, Mangalore – 575028, Karnataka, India.

**Preparation of aqueous extracts:** 50g each of the dry powders were accurately weighed and transferred into separate conical flasks. To each of these flasks 250ml of distilled water was added and macerated for 48 hours, and stirred occasionally, roughly at interval of 6-8 hours. After maceration, the contents of the conical flasks were filtered separately using Whatman filter paper with pore size 11 $\mu$ . The filtrate thus obtained was used in the preparation of the cream formulations and for preliminary phytochemical analysis.

#### Preliminary Phytochemical analysis

##### Detection of carbohydrates

Molisch's test – 1ml of extract was added to 1ml of Molisch's reagent in a glass test tube, 1ml of concentrated sulphuric acid was added along the side. Appearance of violet colour between the two layers indicates presence of carbohydrates.<sup>5</sup>

Iodine test – a drop of iodine solution was added to 1ml of extract. If the colour of the solution turns dark blue-black confirms presence of starch.<sup>5</sup>

##### Detection of Proteins

Biuret test – to 1ml of extract 2ml of 10% sodium hydroxide was added along with 2-3 drops of 1% copper sulphate solution. Formation of blue colour indicates presence of proteins.<sup>5</sup>

##### Detection of lipids

Solubility test – 1ml of the extract was dissolved in test tubes containing water, ethanol, and chloroform. If a layer is observed above the water, partially miscible in ethanol and completely miscible in chloroform then lipids are present.

##### Preparation of cream

##### Detection of alkaloids

Mayer's test – to 1ml of sample 0.5ml of Mayer's reagent was added. Formation of off-white coloured precipitate indicates presence of alkaloids.<sup>5</sup>

##### Detection of flavonoids

Sodium hydroxide test – to 1ml of extract few ml of dilute sodium hydroxide solution was added. Development of sharp yellow colour which disappears on addition of hydrochloric acid indicates presence of flavonoids.<sup>5</sup>

Lead acetate test – to 1ml of extract 0.5ml lead acetate solution was added. Formation of intense yellow colour which disappears when dilute hydrochloric acid was added, indicates presence of flavonoids.<sup>5</sup>

##### Detection of saponins

Foam test – to 2ml of extract add 3-5ml of water in a test tube and shake vigorously and set it aside undisturbed. If a foam is developed at the top of the solution which stays for more than 5 minutes indicates presence of saponins.<sup>5</sup>

##### Detection of tannins

Lead sub-acetate test - to 1ml of sample 3ml of water is added along with a few drops of lead sub-acetate solution. Formation of white-cream coloured precipitate indicates presence of tannins.<sup>5</sup>

Ferric chloride test - to 1ml of sample add 3ml of water and few drops of ferric chloride solution. Development of greenish-black colour indicates presence of tannins.<sup>5</sup>

**Table 1: Formula for the preparation of cream containing *Calendula officinalis* & *Centella asiatica***

Ingredients	Formulation 1 (F <sub>1</sub> )	Formulation 2 (F <sub>2</sub> )	Formulation 3 (F <sub>3</sub> )
<i>Calendula officinalis</i> extract	1 ml	1.5 ml	2 ml
<i>Centella asiatica</i> extract	1 ml	1.5 ml	2 ml
Bees wax	3 g	3g	3g
Liquid paraffin	12 ml	12ml	12ml
Borax	0.2 g	0.2 g	0.2 g
Methyl paraben	0.02g	0.02g	0.02g
Distilled water q. s.	4.8 ml	3.8 ml	2.8 ml

**Procedure:** accurately weighed quantities of the oil phase (i.e., bees wax and liquid paraffin) was taken into an evaporating dish and heated on a water bath to 70°C. In a 100ml glass beaker the aqueous phase i.e., (*Calendula officinalis* and *Centella asiatica* aqueous extracts, borax, methyl paraben and required quantity of distilled water. Stir to dissolve the solid ingredients) was taken and heated on a water bath to 70°C. The aqueous phase was added drop by drop to the oil phase with constant stirring. Once all of the aqueous phase was incorporated into the oil phase, the evaporating dish was taken off the heat and stirred vigorously till the thick cream that had formed cooled down to the room temperature. The cream was transferred into an air tight, wide mouth container.

##### Evaluation of creams

Organoleptic properties: Colour, odour, gloss of the creams was evaluated by visual appearance.<sup>6</sup>

Homogeneity, texture, grittiness: these parameters were evaluated by visual appearance and touch.<sup>6</sup>

After feel – emollient property, slipperiness and the amount of cream left after the application of fixed amount of formulations was checked.<sup>6</sup>

Type of smear - after the application of formulations, the nature of film or smear formed was checked.<sup>6</sup>

Ease of removal - the formulation was applied onto the skin and washed off using water to examine its ease of removal.<sup>6</sup>

pH – stands for potential of hydrogen and is determined by dipping the pH paper into the formulation and comparing the colour change of the paper against the standard.<sup>6</sup>

Spreadability - it is expressed in terms of time (in seconds) taken by two slides to slip off from the formulation placed between the

slides under the application of a fixed load. A small amount of the cream was applied between two slides and excess was scraped off. Time taken by the slide to move 5cm was noted.<sup>6</sup>

Irritancy test – the formulation is applied to an area marked on the skin and kept for an extended period of time.<sup>6</sup>

Type of emulsion – it is checked by 2 methods: - a) by adding a small amount of the formulations to a test tube containing water. b) by mounting a small amount of the formulation onto a glass slide and staining it with a water-soluble dye such as amaranth dye and observing it under a microscope.<sup>6</sup>

Phase separation – the formulations were kept in containers at 25°C – 40°C temperature and checked every 24 hours for 30 days.<sup>6</sup>

## RESULTS

In the present study water-in-oil type creams were formulated by using aqueous extracts of *Calendula officinalis* and *Centella asiatica* dry plant powders as active ingredients in the concentrations of 5%, 7.5% and 10% of each of the extracts, to determine if there is any variation in the three formulations due to increasing concentrations of active ingredients. It was observed that the difference between the three formulations were not that significant and visually they only differed in the colour and texture of the formulations.



Figure 1: Cream formulations F1, F2 and F3 (from left to right).

## Phytochemical Analysis

Table 3: Preliminary Phytochemical Analysis

Phytochemicals	Tests	Result	
		<i>Calendula officinalis</i>	<i>Centella asiatica</i>
Carbohydrates	Molisch's test	-	-
	Iodine test	-	-
Proteins	Biuret test	-	-
Lipids	Solubility test	-	-
Alkaloids	Mayer's test	-	-
Flavonoids	Lead acetate test	+	+
	Sodium hydroxide test	+	+
Saponins	Foam test	+	-
Tannins	Ferric chloride test	+	+
	Lead sub-acetate test	+	+

Irritancy test - The formulations were applied on a 1 square centimetre area on the skin and kept for around 4 hours before removal. The test showed no sign of irritation or inflammation.

Determination of type of emulsion: -

Dilution test – the formulations show water in oil type of emulsion.

Dye test – when stained with amaranth red dye and viewed under microscope it was observed that all the formulations showed red globules in a colourless continuous phase.

Table 2: Organoleptic properties

Parameters	Observations		
	F <sub>1</sub>	F <sub>2</sub>	F <sub>3</sub>
Colour	Off-white	Pale yellow hue	Pale yellow
Odour	Little to no odour	Little to no odour	Little to no odour
Gloss	Shiny	Shiny	Shiny
Texture	Smooth	Textured	Grainy texture
Grittiness	Non-gritty	Non-gritty	Non-gritty

Homogeneity - The formulation was tested for homogeneity by visual appearance and by touch. All the three formulations had good homogeneity.

After feel - Emollience, slipperiness and the amount of residue left after the application of fixed amount of cream was found and all the three formulations had good after feel.

Type of smear - After applying the formulations on the skin, it was found that all the three formulations were slightly greasy and leaving behind an oily layer.

Ease of removal - After applying the formulation on the skin, they were easily removed by washing under tap water.



Figure 2: Aqueous extracts of *Calendula officinalis* (left) and *Centella asiatica* (right)

Phase Separation - The formulation F1 showed no phase separation while the formulations F2 and F3 showed some phase separation after 20 days.

## DISCUSSION

The phytochemical test showed that the aqueous extract of *Calendula officinalis* contains flavonoids, saponins and tannins, while the aqueous extract of *Centella asiatica* contains flavonoids and tannins. The creams thus prepared had good organoleptic properties and other evaluation parameters such as type of smear, after feel, irritancy test etc. The only notable difference was found to be between the colour and texture of the formulations, formulation F1 (containing 5% of aqueous extracts) was off-

white in colour and was of smooth texture while the formulation F2 was pale yellow in colour and textured and formulation F3 had a darker shade of yellow compared to F2 and had a grainy texture. The stability studies showed that formulation F1 was stable even after 30 days while the others showed some evidence of phase separation by day 20. Since the formulation F1 containing 5% each of the aqueous extracts of *Calendula officinalis* and *Centella asiatica*, had a good colour, was smooth in texture and was more stable than the other two formulations F2 and F3 containing 7.5% and 10% of the aqueous extracts respectively.

The extract of *Calendula officinalis* was traditionally used in medicine in various regions of India, China, and Europe, and has significant wound-healing, anti-inflammatory and antioxidant properties,<sup>3</sup> studies conducted on animal models proves the same.<sup>7</sup> The flavonoids present in the extract is believed to be the reason for its medicinal benefits. Studies have shown that the oil obtained from the plant has sun protective properties.<sup>8</sup>

The phytochemicals present in *Centella asiatica* which are responsible for its wound-healing, anti-inflammatory and antioxidant activity was found to be the glycoside called Asiaticoside made up of asiatic acid, madecassoside and madasiatic acid.<sup>4</sup> Various studies have proven that the said phytochemicals obtained from the extracts have good wound healing and anti-inflammatory activity.<sup>9</sup>

By combining the extracts of *Calendula officinalis* and *Centella asiatica* into a formulation, it holds potential to act synergistically to enhance the wound healing and anti-inflammatory effects compared to the individual effects of the plant extracts.

## CONCLUSION

The cream formulations prepared using *Calendula officinalis* and *Centella asiatica* aqueous extracts had good organoleptic and physical characteristics. All the 3 formulations that were prepared had good physical characteristics. However, the formulation F1 containing 5% each of the plant aqueous extracts had the best physical characteristics compared to the other formulations. Formulation F1 had good colour and smooth texture which was more appealing to the eye, also F1 had better stability compared to the formulations F2 and F3.

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